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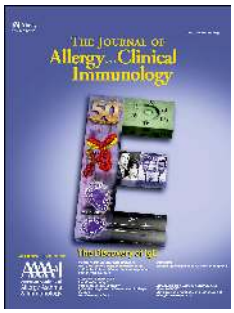

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PII: S0091-6749(16)30893-4

DOI: 10.1016/j.jaci.2016.07.029

Reference: YMAI 12319

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 6 May 2016

Revised Date: 20 July 2016

Accepted Date: 27 July 2016

Please cite this article as: Kennedy EA, Connolly J, O'B Hourihane J, Fallon PG, McLean WI, Murray D, Jo J-H, Segre JA, Kong HH, Irvine AD, Skin microbiome prior to development of atopic dermatitis: early colonization with commensal staphylococci at 2 months is associated with a lower risk of atopic dermatitis at 1 year, *Journal of Allergy and Clinical Immunology* (2016), doi: 10.1016/j.jaci.2016.07.029.

Skin microbiome prior to development of atopic dermatitis: early colonization with commensal staphylococci at 2 months is associated with a lower risk of atopic dermatitis at 1 year

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Funding Sources

ADI is funded by the National Children's Research Centre, Dublin, Ireland. The National Children's Research Centre (NCRC) is the primary funding source for the on-going Cork BASELINE birth cohort. Additional support came from the UK Food Standards Agency (FSA). Work also supported by Intramural Research Program of the NIH National

45 Cancer Institute (EAK, J-HJ, HHK) and National Human Genome Research Institute
46 (JAS).

Abstract

Background

Disease flares of established atopic dermatitis (AD) are generally associated with a low-diversity skin microbiota and *Staphylococcus aureus* dominance. The temporal transition of the skin microbiome between early infancy and the dysbiosis of established AD is unknown.

Methods

We randomly selected 50 children from the Cork BASELINE longitudinal birth cohort for microbiome sampling at three times in the first six months of life, at four skin sites relevant to AD: the antecubital and popliteal fossae, nasal tip, and cheek. We identified ten infants who developed AD and compared them with ten randomly selected control infants with no AD. We performed bacterial 16S ribosomal RNA sequencing and analysis directly from clinical samples.

Results

Bacterial community structures and diversity shifted over time, suggesting that age strongly affects the skin microbiome in infants. Unlike established AD, these infantile AD patients did not have noticeably dysbiotic communities prior to or with disease and were not colonized by *S. aureus*. In comparing patients and controls, infants who had affected skin at month 12 had statistically significant differences in bacterial communities on the antecubital fossa at month 2 compared to infants who were unaffected at month 12. In particular, commensal staphylococci were significantly less abundant in infants

affected at month 12, suggesting that this genus may protect against the later development of AD.

Conclusions

This study suggests that 12-month-old infants with AD were not colonized with *Staphylococcus aureus* before developing AD. Additional studies are needed to confirm if colonization with commensal staphylococci modulates skin immunity and attenuates development of AD.

Clinical Implications or Key Messages

S. aureus colonization was absent in infants with AD. Colonization by commensal staphylococcal species may protect against development of eczema.

Capsule Summary

Staphylococcus aureus colonization does not predate development of AD in infants; colonization by commensal staphylococci may protect against later AD development. Bacterial communities shifted with age during the first year of life.

Key words

Staphylococcus aureus

Atopic dermatitis

Skin

microbiome

Longitudinal birth cohort

16S sequencing

Abbreviations

AD (Atopic Dermatitis)

BASELINE (Babies After Scope: Evaluating the Longitudinal Impact Using Neurological and Nutritional Endpoints)

CUMH (Cork University Maternity Hospital)

INTRODUCTION

Atopic dermatitis (AD) is a common inflammatory skin condition that begins early in life. AD patients with established disease experience frequent colonization and increased infections with *Staphylococcus aureus* as well as potentially life-threatening eczema herpeticum with herpes simplex virus. The hygiene hypothesis relates the development of atopic disorders (AD, allergic rhinitis, and asthma) to reduced microbial exposure at a young age[1]. Epidemiological studies examining the incidence of asthma have linked exposure to farming environments to lower rates of allergic disorders[2-4]. However, the potential role of microbe exposure in early childhood to the development of AD and the subsequent atopic march towards the development of allergic rhinitis and asthma remains to be elucidated.

There is significant interest in the potential effects of microbes on the development of skin immunity as well as disease [5-9]. Recent work in mice has shown that cutaneous exposure to commensal bacteria early in life can induce tolerance to these microbes[6]. Given these epidemiologic associations between environmental exposure and development of atopic diseases, we investigated the skin microbiome in a birth cohort. We analyzed bacterial 16S rRNA gene sequences from swabs collected from four skin sites in infants in a birth cohort (Babies After Scope: Evaluating the Longitudinal Impact Using Neurological and Nutritional Endpoints/BASELINE) at three different time points to determine if the differences in the skin microbiome were associated with AD development.

METHODS

Study subjects

The Cork Babies After Scope: Evaluating the Longitudinal Impact Using Neurological and Nutritional Endpoints (BASELINE) birth cohort study is the pediatric follow-on from the Cork Centre for the Screening for Pregnancy Endpoints (SCOPE) study[10, 11]. The Cork BASELINE birth cohort study recruited within a white Irish population in Cork, Ireland from August 2009 to October 2011. These women were subject to the inclusion criteria of the SCOPE study: low-risk primigravidous mothers with singleton pregnancies, who delivered at or near term. Maternal consent was obtained at 20 weeks gestation, and verified at delivery. Ethical approval for the Cork BASELINE birth cohort Study was granted by the Clinical Research Ethics Committee of the Cork Teaching Hospitals, ref ECM 5 (9) 01/07/2008. The BASELINE study is registered with the United States National Institutes of Health Clinical Trials Registry (http://www.clinical trials.gov), ID: NCT01498965.

Clinical diagnosis of Atopic Dermatitis

All infants were assessed at birth, and at 2, 6, 12, and 24 months of age. Assessment included parental questionnaires and physical exam. Screening questions specific for atopic dermatitis were included in the questionnaires administered at 2, 6, and 12 months. AD was diagnosed (at 6, 12, and 24 months) by experienced healthcare personnel using the UK Working Party diagnostic criteria[12-14]. When AD was present, the SCORAD (SCORing Atopic Dermatitis) clinical tool was used to assess severity[15, 16]. The

Nottingham Eczema Severity Score (NESS) was also used to assess AD severity at 12 months[17]. Demographic data and clinical details are shown in Table 1 and Table E1.

Filaggrin (FLG) genotyping

Cord blood samples were collected at birth and stored for analysis. *FLG* genotyping was carried out on all study subjects with testing for the four most common Irish/European mutations as previously described[18]. None of the subjects in this study were found to have *FLG* mutations.

Sampling for microbiome analysis

We randomly selected 50 infants from the birth cohort and obtained skin swabs at day 2, month 2, and month 6. Skin samples and negative controls were collected using pre-moistened swabs as previously described[19]. After all infants had been assessed at 1 year, ten infants with clinical AD at months 2, 6 and/or 12 were selected for analysis as 'AD patients'. Healthy controls were ten infants without AD at any study time points selected at random. Sample sites were selected based on the presentation of AD at different ages. Cheeks (Ch) are a site of AD predilection in infants, and the nasal tip (Nt) is typically spared. Antecubital fossae (Af) and popliteal fossae (Pf) are typical sites of AD predilection in children and adolescents.

Sample processing/sequencing

16S rRNA V1-V3 sequencing was performed on swab samples as previously described[19]. Swabs were incubated in Yeast Cell Lysis Solution (Epicenter MasterPure kit, MPY80200) and Ready-Lyse Solution (Epicenter R1802M) for 1 hour at 37°C. Two 5-mm stainless steel beads (Qiagen) were added and processed in a TissueLyser (Qiagen) for 2 min at 30 Hz. The solution was treated with MPC Protein Precipitation Reagent (Epicenter MasturePure kit MPY80200) to remove cellular debris. Subsequent steps were performed using the PureLink Genomic DNA kit (Invitrogen). Barcoded primers flanking V1 (27F, 59-AGAGTTTGATCCTGGCTCAG-39) and V3 (534R, 59-ATTACCGCGGCTGCTGG-39) were used for PCR. PCR products were purified using the Agencourt AMPure XP Kit (A63880) and quantitated using the Quant-iT dsDNA high-sensitivity assay kit (Invitrogen, Q33120); equivalent amounts of these PCR products were pooled, purified with a Qiagen MinElute column (Qiagen, 28004) into 30 μL TE, and sequenced at the NIH Intramural Sequencing Center on a 454 GS FLX (Roche) platform. Reagents and collection procedure controls were tested and demonstrated no significant background contamination.

Data analysis

Sequences were preprocessed using mothur version 1.35.1[20]. Briefly, 454 flowgram data were trimmed and denoised and chimera-checking was completed using the mothur implementation of UCHIME[21]. Sequences were classified using the Ribosomal Database Project naïve Bayesian classifier[22]. **Sequences classified as Chloroplast or Mitochondria were discarded.** Site-specific definition of operational taxonomic units (OTUs, or groups of sequences that share a specific level of similarity) and downstream

analyses was performed in mothur. Within the samples from each time point or site, pairwise distances were calculated and OTUs defined at 97% nucleotide similarity. Within-sample (Shannon diversity) and between-sample (theta index) measurement were performed based on these OTU definitions with subsampling to 1000 sequences per sample[23]. Rarefaction curves level off by this value, suggesting adequate sequencing coverage; any samples with fewer than 1000 sequences after preprocessing were removed from analysis (Fig E1). Differentially abundant OTUs were detected using the metastats command in mothur.

The sequences classified to the *Staphylococcus* genus by the RDP naïve Bayesian classifier were then placed on a phylogenetic reference tree using "-keep-at-most 1000 max-pitches 1000". Taxonomy was assigned using the guppy program in pplacer[24] with a likelihood cutoff set to 0.65 as previously described[19].

Statistics

All data analysis was performed in R; results are presented as mean ± SEM unless otherwise indicated. The 95% confidence intervals were estimated. Post-hoc tests (i.e. pairwise comparisons in AMOVA) were adjusted using a Bonferroni correction. For detection of differentially abundant OTUs, metastats results are filtered for OTUs with a mean abundance of 0.05% or greater and p-values calculated using an FDR adjustment.

RESULTS

Site-specific bacterial colonization patterns

Since different skin microenvironments and anatomic regions harbor distinct microbial communities in adults and older children[25, 26], we initially compared the major bacterial taxa present on the four sites on infants. Relative abundances of these bacterial taxa showed differences between the two facial sites (Ch and Nt) and the extremity sites (Af and Pf) (Fig E2). Calculation of differentially abundant species between the site types confirmed that *Staphylococcus* spp. were relatively more abundant on extremity sites at all time points, and *Gemella* spp. were relatively more abundant on facial sites. Other taxa were only differentially represented at some time points, with facial sites in *Propionibacterium* spp. at day 2 and *Streptococcus* spp. at later time points (Table E2).

We validated these findings with biodiversity calculations, examining samples from each time point. We analyzed how similar the bacterial community structures were between the samples using the theta similarity index, which accounts for both the presence and proportion of bacterial species[23]. A theta index value of 1 indicates that the two bacterial communities have identical structures; a value of 0 indicates maximally dissimilarity. In principal coordinates analyses (PCoA) based on these theta values, samples that are more similar to each other cluster more closely together. At each time point, the facial site samples clustered together (AMOVA p-value > 0.05) but distinctly separate from the extremity site samples (p-value < 0.006). The extremity sites clustered together at day 2 and month 2, but had different centroids at month 6 (p-value=<0.006) (Fig 1 and Fig E3).

Changes in bacterial colonization over time

Skin microbiomes differ between children and adults; however, studies with longitudinal skin sampling in infants are infrequent[27, 28]. Alterations in the skin bacterial abundances at the different sampling time points were apparent in our cohort (Fig E4). For each skin site, the bacterial community structures showed striking shifts based on sampling timepoint (Fig 2AB, Fig E5). At both extremity sites, the samples clustered separately between day 2 and month 6 (AMOVA p-value = 0.024 for Af, 0.003 at Pf). For both facial sites, day two and month 6 samples clustered significantly as well ($p<0.003$ for each), and between month 2 and month 6 ($p<0.003$ at Ch, $p=0.06$ at Nt).

To examine interpersonal variation, we calculated the mean similarity between samples at a single site and time point. For both facial sites, bacterial communities between subjects were most similar at month 6, converging to a more common bacterial population across subjects. Extremity sites did not present this same pattern; instead the most similar bacterial community structures were observed at month 2 (Fig 2C).

We analyzed the bacterial diversity of all samples, using the Shannon diversity index (a higher value signifies more taxonomic groups and/or a more even distribution of these groups). At each time point, diversity was similar between Af, Ch, and Nt (Wilcoxon Rank-Sum test p-value > 0.05; Fig 3, Table E3). Pf had a substantially altered pattern, significantly different from the other sites at all time points, except Af at day 2. At the facial sites, bacterial diversity increased significantly over the time studied (Wilcoxon Rank-Sum test p-value < 0.001 for each site between Day 2 and Month 6; Table E3).

Combined with the increasingly similar bacterial community structures on the face, this suggests that over time the microbial population converges and stabilizes at facial sites. Samples from the antecubital fossa also significantly increased in diversity between the time points (p=0.033).

Colonization of antecubital fossa with commensal staphylococci at month 2 is associated with decreased incidence of AD at 1 year

To identify any bacterial differences associated with AD in this cohort, we compared infants who developed AD at any time within the first year of life versus controls for each site and sampling time. At all time points, the bacterial community structures of infants who developed AD at any time within the first year of life did not cluster separately from control infants, and no significant differences in Shannon diversity were identified between the groups. Since the patients had clinical disease presenting at different time points (Table E1), we also compared samples based on whether the subjects presented with disease at each time point. Overall, there was almost no distinction between affected and unaffected samples in within- or between-sample diversity, either before or at the time point when the patients were affected (Tables E3, E4).

Interestingly, the month 2 Af samples demonstrated statistically significant clustering, grouped by those infants that went on to be affected at month 12 in this study (AMOVA p-value = 0.003). OTU-based analysis suggested that a single OTU was differentially abundant between the groups; this OTU was classified as *Staphylococcus* (Fig 4A). When considering all sequences classified to the *Staphylococcus* genus, the relative abundances

were significantly different between the two groups, with subjects that went on to be affected colonized by significantly less staphylococci (mean 0.065, 95% CI: 0.035-0.094) as compared to those that went on to be unaffected (mean 0.495, 95% CI 0.458-0.531). ($p < 0.003$ for Wilcox rank-sum test) (Fig 4B).

Given the specific association between *S. aureus* and AD flares, we classified the *Staphylococcus* sequences to the species level; in these samples, the most prevalent species were *S. epidermidis* and *S. cohnii (*Fig 4C*)*. In contrast to older patients with AD[29], essentially no *S. aureus* sequences were present in the samples in our cohort, even at the sites and times that patients were affected (Fig E6-7). There were no statistically significant differences within individual *Staphylococcus* spp. levels in the month 2 Af samples between the later-affected and later-unaffected samples.

Birth Method and Feeding Method Have Little Effect on Skin Microbiota

Differences have previously been reported between the skin microbiota of infants born by C-section versus vaginal birth[28]. We investigated whether birth method was associated with differences in skin microbiota in our cohort. There was no clustering of samples at any site or time point based on birth method, except Af samples at day 2 (Fig E8AB). No statistically significant differences in skin colonization based on birth method were observed at the earliest timepoint in this cohort, the second day of life. Shannon diversity was similar between the two birth methods as well (Fig E8C). Feeding method has been associated with differences in the intestinal microbiome composition of infants[30, 31].

316 However, feeding method – breast, formula, or combination – and gender did not affect
317 skin bacterial colonization patterns in this cohort (Tables E3, 4)
318
319 **Discussion**
320 While infections with *S. aureus* and herpes simplex virus can complicate the
321 course of established AD, the role of microbes in the etiology, genesis and pathogenesis
322 of AD remains unclear. Recent murine studies have shown that cutaneous microbes can
323 influence the development of skin immunity and disease[5, 6, 9]. Determining if cutaneous
324 microbes play a role in the initiation of AD could provide an opportunity to reduce the
325 development of atopic disorders. To investigate the skin microbiome in infants prior to
326 the development of AD, we used 16S rRNA gene sequencing of skin samples from a
327 birth cohort and determined that shifts occur in the skin microbiome over the first six
328 months of life, with site-specific bacteria communities changing in composition and
329 diversity over time. We also identified a difference in staphylococcal colonization at a
330 site of AD predilection that predates the presentation of disease, with patients who went
331 on to be affected at a later date colonized by fewer *Staphylococcus* spp. Birth method and
332 feeding method did not appear to affect skin bacterial communities at the sites and time
333 points studied in this cohort, but other studies are needed to confirm these findings.
334 Prior studies of human skin have shown that skin microbial communities are site-
335 specific[32, 33]. While there is heterogeneity of bacterial communities across the skin
336 surface, specific skin sites in different individuals often share common patterns of
337 bacterial composition. This biogeography of the skin microbiome has been observed in
338 older children and adults [26, 34]. In previous infant skin microbiome studies, site-specific

differences were not evident in the first 3 months of life because infants were studied at a single timepoint immediately after delivery or were sampled at a single timepoint and analyzed in age cohorts of 1-3, 4-6, and 6-12 months[27, 28]. The present study differed by sampling the same cohort of infants over a six-month interval (day 2, month 2, and month 6) and observed site-specific differences as early as the second day of life, a timepoint not previously investigated. The bacterial diversity of one skin site, the popliteal fossa, shifted at time points differentially from the three other sites studied. Since this specific skin site has not been examined in a cohort this young, the results may be related to a unique aspect of infant skin physiology and/or exposure, or specific to this cohort. Interestingly, the body site differences in bacterial communities also reflect observed site differences in immune cell density and composition from human skin[35-38]. Investigating site-specific differences in host-microbial interactions may enhance our understanding of the predilection of certain skin regions for dermatologic diseases.

In addition to the biogeography of the skin microbiome, skin bacterial communities can shift significantly during different periods of the life cycle, such as puberty[26]. The physiology of infant skin changes over the first year of life with alterations in stratum corneum hydration, skin pH, and sebum production[39]. In this study, the shifts in skin bacterial communities in the first months of life were the inverse of skin microbiome alterations that have been observed later in childhood. The increasing Shannon diversity observed in the first year of life in this infant cohort supports previous work that showed increased evenness, or similar numbers in each taxa, in bacterial communities from three skin sites in a cross-sectional study [27]. During puberty, significant shifts in skin bacterial communities likely reflect the changes in skin

physiology and systemic hormones[26]. The changes in the skin microbiome observed in these infants potentially reflect the influences of waning maternal hormones as well as the continued development of infant skin. For example, lipophilic *Propionibacterium* are relatively abundant on the facial sites at neonatal day 2, but decrease substantially at later time points. This corresponds to the high sebaceous activity triggered by maternal hormones in the first days of life, which wane significantly in the weeks after birth[40]. These findings lead to additional questions, including whether neonatal skin disorders, e.g. cephalic pustulosis (aka neonatal acne), attributed to maternal hormones potentially may also be affected by alterations in skin bacteria.

A previous study in mice reported that developing antigen-specific tolerance to commensals depends on early colonization, suggesting that there is a 'critical window' for inducing regulatory T cells that prevent a later inflammatory response to these bacteria[6]. Scharschmidt et al. showed that application of a commensal species of *Staphylococcus* on neonatal skin induced these immunomodulatory effects. The relatively low abundance of pathogenic staphylococcal species on the antecubital fossa of two-month-old infants who later had AD at twelve months of age is intriguing in the context of this prior work in mice. Whether cutaneous exposure to commensal staphylococci during early infancy may have a similar effect remains unknown and further investigation is needed to understand if this may influence the development of AD. The absence of *S. aureus* at AD lesions in this cohort was somewhat surprising, given that this species is associated with AD [29, 41-43]. A culture-based analysis of infant skin demonstrated *S. aureus* colonization in approximately 21% of AD lesions among infants in their first year of life[44]. The differences may be related to inherent differences in the study populations

and/or the severity of sampled skin lesions between the study groups.

Differences in birth method have been studied in relation to the incidence of atopy and to the neonatal skin microbiome[28, 45]. An earlier study showed skin microbiome differences based on birth method in neonates sampled a few minutes after delivery. The small sample size and rare number of Caesarean deliveries in the current study potentially contribute to the lack of statistically significant differences between the skin microbiota of infants born vaginally or by Caesarean at the earliest time point in this study, day 2 of life. While this study analyzed different sites over a longer time frame than the previous work, a larger study would be needed to address this question. Birth method may determine skin colonization very early in life; however, environmental exposures and skin physiology may predominate in shaping bacterial communities after this initial delivery. The skin barrier and *FLG* mutations are additional aspects of skin physiology that have been studied in relation to atopy. While approximately 10% of subjects in BASELINE publications and the Irish population have *FLG* mutations, the current cohort had fewer *FLG* mutations than expected due to sampling effects. Since a large proportion of patients with atopic dermatitis do not carry *FLG*-null alleles, the results in the current cohort avoid the potential effects of *FLG* mutations and remain relevant to AD. With interest in the potential immunological effects of neonatal exposures to skin microbes[6, 46], characterizing the early skin microbiome in neonates with and without *FLG* mutations and the timeframe for possible development of immunotolerance would be of significant clinical importance.

There are increasing efforts to understand the potential relationship between the skin microbial landscape and the development of skin immunity and human disease.

Early studies of the skin microbiome will identify possible associations between specific microbes and human health and disease but need extensive further research will be required to unravel the pathophysiology and key mechanisms involved. Longitudinal sampling of the same individuals as internal controls, and the initiation of sampling soon after birth were features of this study that improve the ability to identify distinct microbial patterns that could provide insight into the skin microbial milieu prior to the development of skin disease. As a result, we were able to define the site-specificity and the longitudinal shifts of the skin microbiome in the first six months of life, as well as the difference in relative abundances of commensal staphylococci prior to the development of AD. Additional investigations are needed to test whether site-specific differences in skin microbes influence the development of atopic dermatitis.

Acknowledgements

We thank Sean Conlan and Weng-Ian Ng for their underlying efforts; NISC Comparative Sequencing Program for sequencing; and Mark C. Udey for discussions. This work utilized the computational resources of the NIH HPC Biowulf cluster (http://hpc.nih.gov).

428

429 **Table 1. Demographic data for study subjects**

430

		Healthy controls	AD patients
Female:Male		6:4	5:5
C-section:Vaginal		1:9	3:7
BF:FF:C*		2:3:5	1:2:7
Rural:Urban		5:5	4:6
Pet:No pet		5:5	3:7
Emollient use (Month 2) – Y:N		2:8	6:4
Bathing frequency (Month 2) – ≤weekly : > weekly		5:5	6:4
Antibiotic use (Month 2) – Y:N		1:9	1:9
Antibiotic use (6 months) – Y:N		5:5	3:7
TEWL	**Day 2**	9.668 ± 0.776	9.749 ± 0.618

	Month 2	10.402 ± 1.619	11.124 ± 2.135
	Month 6	11.412 ± 2.149	10.08 ± 1.342

***BF – breast-fed exclusively; FF – formula-fed exclusively; C – combination feeding**

Figure legends

Fig 1: Site-specificity of bacterial community composition

All samples at day two clustered by principal coordinates analysis based on theta similarity coefficients. At day two, Af and Pf clustered together (AMOVA p-value=1), as did Ch and Nt (p=1), but each clustered distinctly from the other site pair ($p < 0.006$). P-values adjusted with Bonferonni correction (n=6)

Fig 2: Skin microbial communities on infants undergo site-specific shifts in composition with age.

(a) Antecubital fossa samples clustered by principal coordinates analysis based on theta similarity coefficients. Using AMOVA, samples clustered distinctly between day 2 and month 6 (p=0.024), but not significantly between month 2 and month 6 (p=0.12) or between day 2 and month 2 (p=0.21)*

(b) Cheek samples clustered by principal coordinates analysis based on theta similarity coefficients. Using AMOVA, samples clustered significantly between day 2 and month 6 ($p < 0.003$), between month 2 and month 6 ($p < 0.003$), but not between day 2 and month 2 (p=0.102)*

(c) Mean theta similarity coefficients of comparisons within samples of each time point. Higher theta values signify greater similarity (Wilcoxon Rank-Sum *: $p<0.05$, **: $p<0.01$, *** $p<0.001$).

Post-hoc p-values adjusted with Bonferonni correction (n=3)

Fig 3: Changes in bacterial biodiversity with age

Mean Shannon diversity at each time point; Shannon diversity is calculated based on richness and evenness of taxa within the community. (Wilcoxon Rank-Sum *: $p<0.05$, **: $p<0.01$, *** $p<0.001$).

Fig 4: Antecubital fossa microbial community differences predate AD presentation.

(a) Month 2 antecubital fossa samples by principal coordinates analysis of theta similarity coefficient. Samples clustered by those that went on to be affected at month 12 and those that were unaffected at month 12 (AMOVA p-value = 0.003).

(b) Relative abundance of major taxa; subjects that went on to be affected at month 12 had significantly lower proportions of *Staphylococcus* than those that went on to be unaffected (Wilcoxon Rank-Sum p-value = 0.008)

(c) Relative abundance of staphylococcal species. *S. aureus* was essentially absent in these communities.

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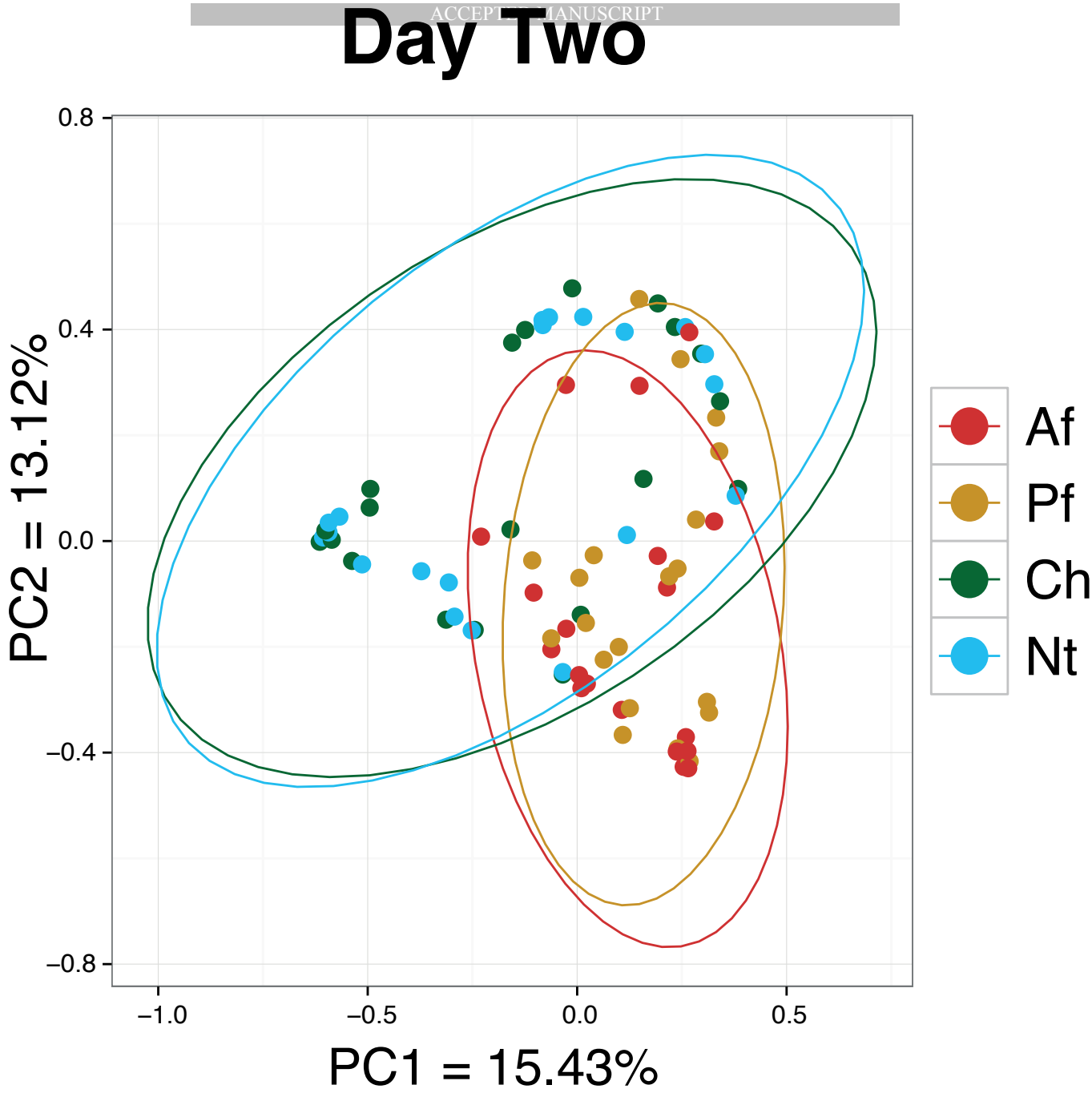
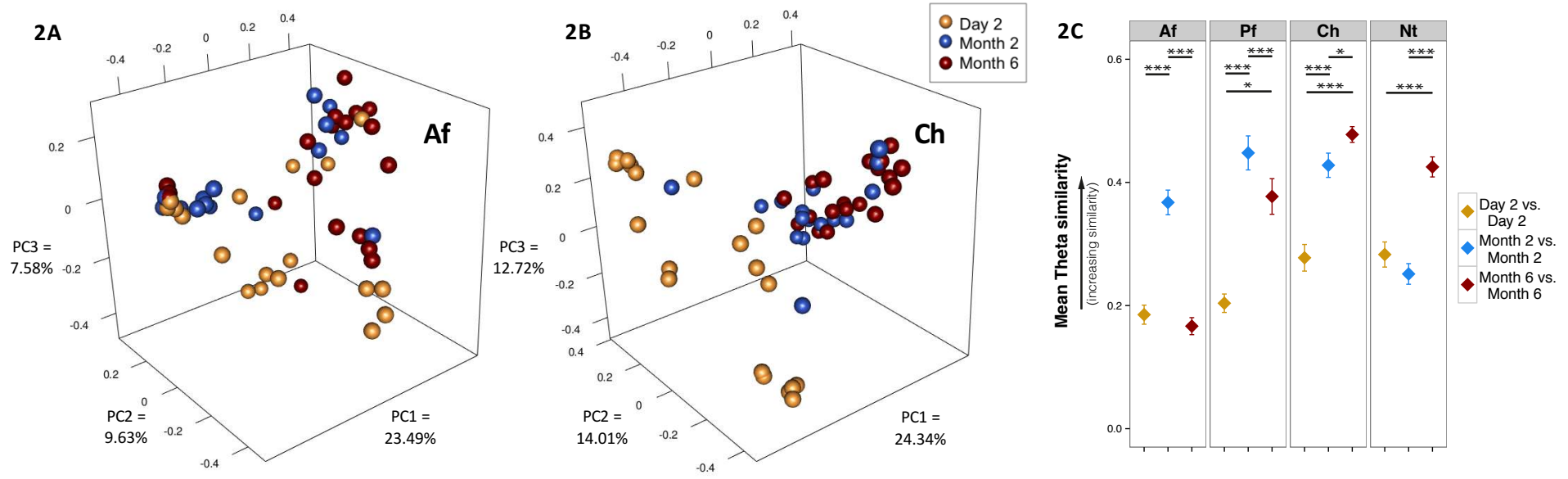
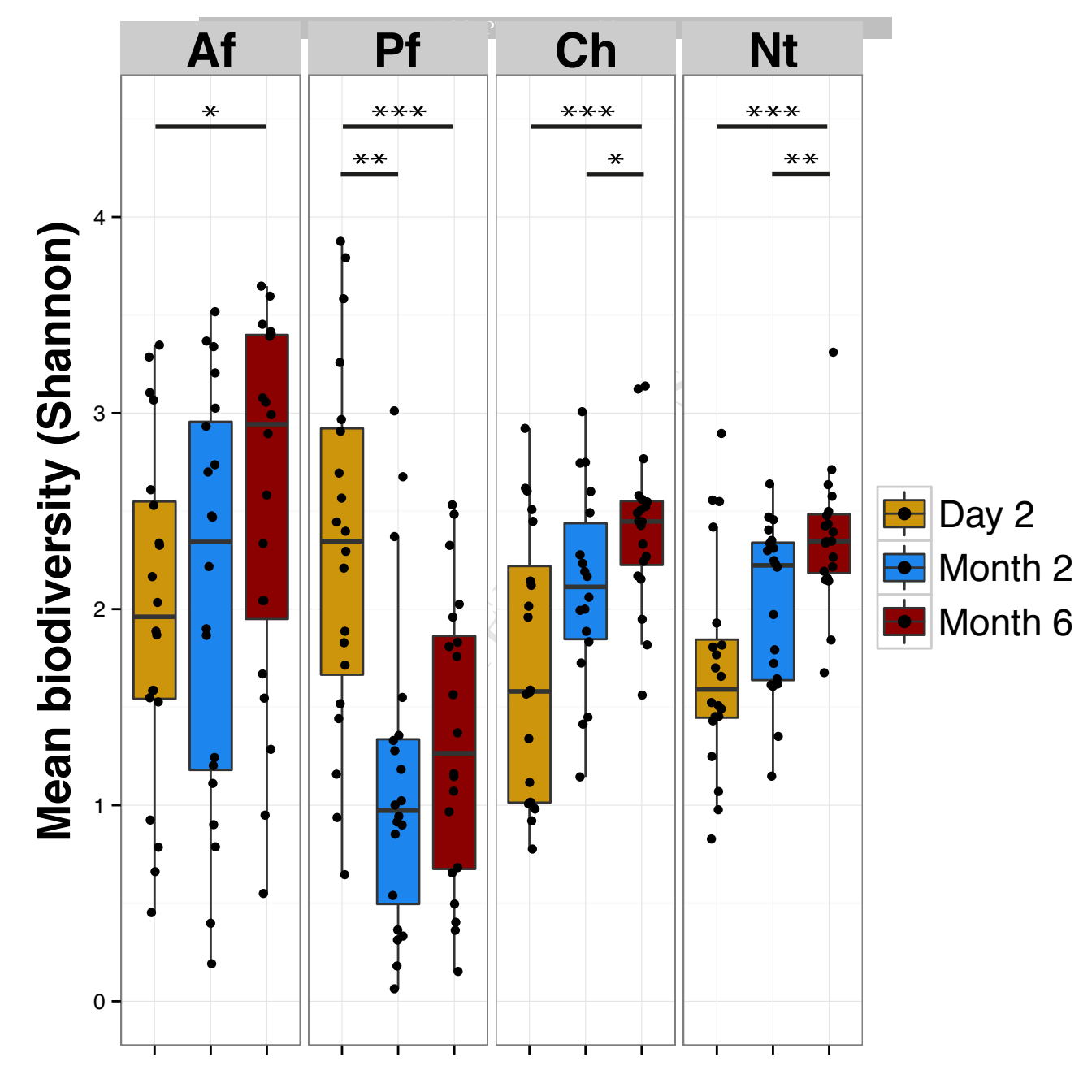



2A
Af
PC3 = 7.58%
PC2 = 9.63%
PC1 = 23.49%
2B
Ch
PC3 = 12.72%
PC2 = 14.01%
PC1 = 24.34%
Day 2
Month 2
Month 6
2C
Af
Pf
Ch
Nt
Mean Theta similarity
(increasing similarity)
Day 2 vs. Day 2
Month 2 vs. Month 2
Month 6 vs. Month 6


Af
Pf
Ch
Nt
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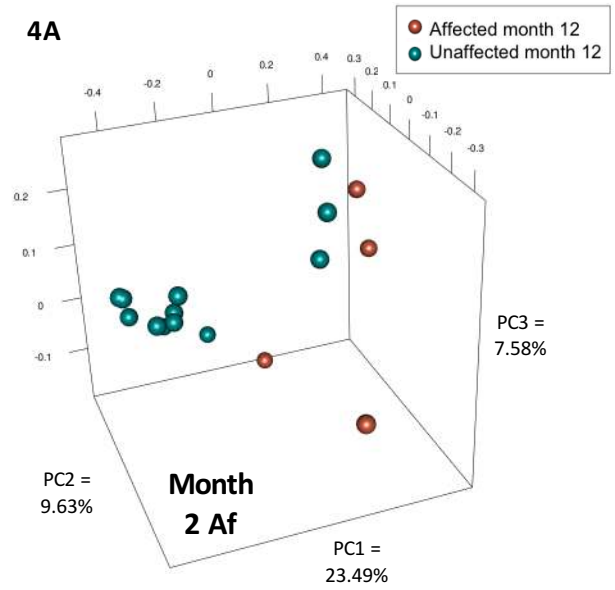
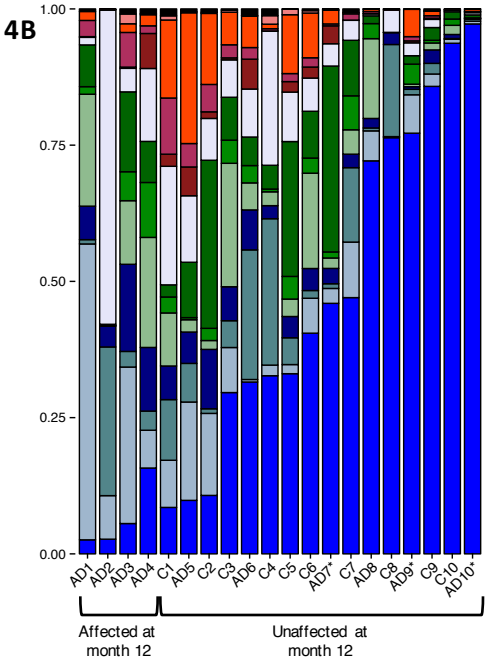
Mean biodiversity (Shannon)
0
1
2
3
4
Day 2
Month 2
Month 6


4A
Affected month 12
Unaffected month 12
PC3 =
7.58%
PC2 =
9.63%
Month
2 Af
PC1 =
23.49%


4B
1.00
0.75
0.50
0.25
0.00
Other
Proteobacteria (other)
Alphaproteobacteria
Betaproteobacteria
Gammaproteobacteria
Bacteroidetes
Actinobacteria (other)
Corynebacteriaceae
Propionibacteriaceae
Firmicutes (other)
Clostridia
Streptococcaceae
Staphylococcaceae
AD1 AD2 AD3 AD4 C1 AD5 C2 C3 AD6 C4 C5 C6 AD7* C7 AD8 C8 AD9* C9 C10 AD10*
Affected at month 12
Unaffected at month 12

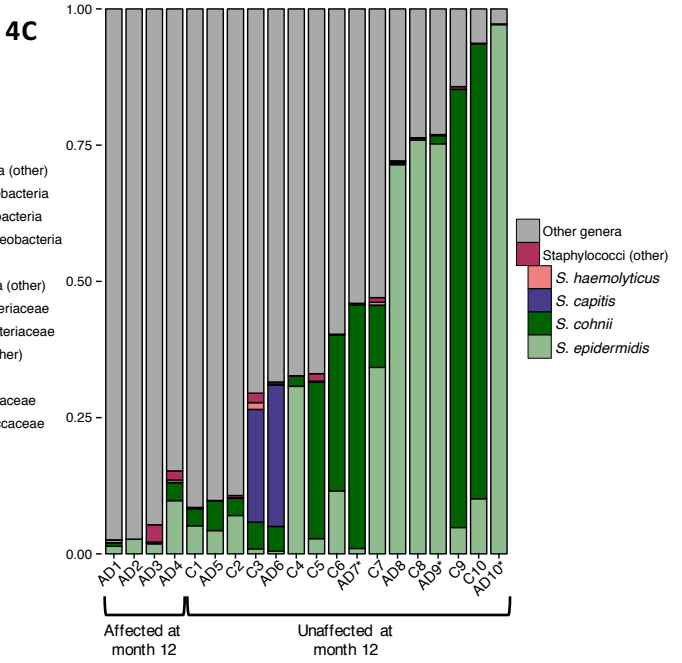

4C
1.00
0.75
0.50
0.25
0.00
Other genera
Staphylococci (other)
S. haemolyticus
S. capitis
S. cohnii
S. epidermidis
AD1 AD2 AD3 AD4 C1 AD5 C2 C3 AD6 C4 C5 C6 AD7* C7 AD8 C8 AD9* C9 C10 AD10*
Affected at month 12
Unaffected at month 12

Table E1: Metadata for each subject

Subject ID	Subject type	Presence of eczema				Mode of delivery	Method of feeding	Pet Exposure during pregnancy and 1st two months of life	2 month Urban/Rural	2 month - frequency of washing	2 month - emoillent use	2 month - antibiotic use	6 month - antibiotic use
		Month 2 (affected site)	Month 6 (affected site)	Month 12 (affected site)	Month 24 (severity)								
AD1	AD patient	-	+ (Ch, Pc)	+ (skinfold at front of both ankles)	SCORAD 19 NESS mild	elective c section	Formula Fed only	No	Rural	weekly	No	No	No
AD2	AD patient	-	-	+ (back: under both shoulders)	-	normal vaginal delivery	Breast Fed (received occasional formula)	Yes (cat)	Rural	2-3 times/week	No	No	No
AD3	AD patient	-	-	+ (front of left knee)	NESS mild	normal vaginal delivery	Breast Fed (received occasional formula)	No	Urban	weekly	No	No	No
AD4	AD patient	-	+ (Ch, Pc)	+ (cheeks, forearms, Rt thigh)	NESS moderate	normal vaginal delivery	Formula Fed only	No	Rural	weekly	Yes	No	Yes (2 times)
AD5	AD patient	-	+ (Pc)	-	-	normal vaginal delivery	Breast fed for 10 days only	Yes (fish)	Urban	2-3 times/week	Yes	No	No
AD6	AD patient	-	+ (Ch)	-	-	elective c section	Breast fed for 41 days only	No	Urban	weekly	Yes	No	No
AD7*	AD patient	+ (?)	-	-	-	normal vaginal delivery	Combination feeding	No	Rural	weekly	Yes	No	Yes (1 time)
AD8	AD patient	-	+ (Ac, Pc)	-	-	elective c section	Breast fed for 13 days only	No	Urban	2-3 times/week	No	No	No
AD9*	AD patient	+ (?)	+ (Ch, Ac)	-	SCORAD 36 NESS moderate	normal vaginal delivery	Breast fed for 42 days	Yes (dog)	Urban	daily	Yes	No	No
AD10*	AD patient	+ (?)	+ (Ch)	-	NESS mild	normal vaginal delivery	Breast Fed Only	No	Urban	weekly	Yes	Yes (1 time)	Yes (same as 2 months)
C1	control				-	normal vaginal delivery	Breast Fed Only	Yes (dog)	Rural (farm)	every 10days	No	No	Yes (1 time)

C2	control				-	normal vaginal delivery	Formula Fed only	Yes (fish)	Urban	daily	No	Yes (IV)	Yes (same as 2 months)
C3	control				-	vacum delivery	Combination feeding	No	Urban	2-3 times/week	Yes	No	No
C4	control				-	normal vaginal delivery	Combination feeding	No	Rural	2-3 times/week	No	No	Yes (1 time)
C5	control				-	normal vaginal delivery	Combination feeding	No	Rural	weekly	No	No	Yes (1 time)
C6	control				-	normal vaginal delivery	Combination feeding	No	Rural	2-3 times/week	No	No	No
C7	control				no data	emergency c section	Formula Fed only	Yes (dog)	Rural	weekly	Yes	No	Yes (1 time)
C8	control				-	normal vaginal delivery	Breast Fed (received occasional formula)	Yes (dog)	Urban	3-5 times/week	No	No	No
C9	control				-	normal vaginal delivery	Formula Fed only	Yes (dog, cat, fish)	Urban	weekly	No	No	No
C10	control				-	vacum delivery	Breast Fed Only	No	Urban	weekly	No	No	No

Table E2: Metastats by site type

Time point	OTU	Mean - Face	Variance - Face	SE - Face	Mean - Extremity	Variance - Extremity	SE - Extremity	p-value	p-value (FDR-corrected)	OTU size	OTU classification	Higher in
Day 2	Otu0002	0.079975	0.009553	0.015454	0.2406	0.070343	0.041935	0.000999	0.00724275	65073	Staphylococcus(100)	Extremity
Day 2	Otu0003	0.247075	0.067132	0.040967	0.018725	0.003489	0.009339	0.000999	0.00724275	48068	Gemella(100)	Face
Day 2	Otu0006	0.0019	1.70E-05	0.000653	0.065875	0.026238	0.025611	0.000999	0.00724275	21437	Streptococcus(100)	Extremity
Day 2	Otu0009	0.040075	0.003209	0.008956	0.0063	0.000311	0.002786	0.000999	0.00724275	9229	Rothia(100)	Face
Day 2	Otu0005	0.097675	0.049571	0.035203	0.004125	0.000123	0.001751	0.002997	0.01655486	29524	Streptococcus(100)	Face
Day 2	Otu0013	0.00375	6.40E-05	0.001265	0.02245	0.001789	0.006689	0.003996	0.01655486	5615	Corynebacterium(100)	Extremity
Day 2	Otu0023	0.00055	2.00E-06	0.00024	0.009675	0.000807	0.004491	0.003996	0.01655486	1911	Enterococcus(100)	Extremity
Day 2	Otu0001	0.2323	0.054002	0.036743	0.109125	0.019272	0.02195	0.00999	0.03621375	69564	Propionibacterium(100)	Face
Day 2	Otu0029	0.000825	5.00E-06	0.000368	0.007675	0.000346	0.002941	0.013986	0.045066	1609	Corynebacterium(100)	Extremity
Month 2	Otu0001	0.056395	0.006899	0.013474	0.4912	0.11718	0.054125	0.000999	0.0033966	114867	Staphylococcus(100)	Extremity
Month 2	Otu0002	0.276474	0.036481	0.030984	0.05235	0.009377	0.015311	0.000999	0.0033966	49332	Streptococcus(100)	Face
Month 2	Otu0006	0.052895	0.002803	0.008589	0.00955	0.000283	0.002658	0.000999	0.0033966	9053	Gemella(100)	Face
Month 2	Otu0008	0.057289	0.006236	0.01281	0.002875	4.60E-05	0.001071	0.000999	0.0033966	7726	Veillonella(100)	Face
Month 2	Otu0010	0.033737	0.00107	0.005307	0.009825	0.000363	0.003011	0.000999	0.0033966	7201	Veillonella(100)	Face
Month 2	Otu0015	0.027974	0.002144	0.007511	0.002725	6.00E-05	0.001221	0.000999	0.0033966	5623	Prevotella(100)	Face
Month 2	Otu0018	0.035184	0.005951	0.012514	0.00085	3.00E-06	0.000283	0.000999	0.0033966	4486	Porphyromonas(100)	Face
Month 2	Otu0025	0.000632	1.00E-06	0.000157	0.011575	0.000931	0.004824	0.000999	0.0033966	1858	Paracoccus(56)	Extremity
Month 2	Otu0029	0.000632	1.00E-06	0.000143	0.0092	0.000824	0.004539	0.000999	0.0033966	1315	Kocuria(64)	Extremity
Month 2	Otu0030	0.011789	0.000339	0.002988	0.00075	4.00E-06	0.00032	0.000999	0.0033966	1307	Actinomyces(100)	Face
Month 2	Otu0007	0.058237	0.019089	0.022413	0.00175	3.30E-05	0.00091	0.001998	0.003996	7949	Prevotella(99)	Face
Month 2	Otu0009	0.032737	0.004386	0.010743	0.002525	6.50E-05	0.001274	0.001998	0.003996	7221	Simonsiella(100)	Face
Month 2	Otu0016	0.027816	0.000981	0.005081	0.006275	0.000384	0.0031	0.001998	0.003996	4977	Rothia(100)	Face
Month 2	Otu0022	5.30E-05	0	5.30E-05	0.011975	0.002619	0.008092	0.001998	0.003996	2110	Bacteroides(100)	Extremity
Month 2	Otu0027	0.011158	0.000613	0.004016	6.00E-04	3.00E-06	0.00027	0.001998	0.003996	1635	Prevotella(100)	Face
Month 2	Otu0031	0.000895	3.00E-06	0.000289	0.00765	0.000156	0.001974	0.001998	0.003996	1303	Chryseobacterium(100)	Extremity
Month 2	Otu0032	0.000605	5.00E-06	0.000359	0.006375	0.000199	0.00223	0.001998	0.003996	1088	Rhizobium(84)	Extremity
Month 2	Otu0005	0.071789	0.030372	0.028271	0.00285	8.00E-05	0.001415	0.004995	0.00893842	10639	Corynebacterium(100)	Face
Month 2	Otu0021	0.000342	1.00E-06	0.000193	0.01825	0.003418	0.009244	0.004995	0.00893842	2248	Porphyromonas(100)	Extremity
Month 2	Otu0003	0.010026	0.000632	0.004079	0.076875	0.032224	0.028383	0.005994	0.00970457	17518	Anaerococcus(100)	Extremity
Month 2	Otu0012	0.000447	4.00E-06	0.000322	0.021275	0.008443	0.014529	0.005994	0.00970457	6278	Bacteroides(100)	Extremity
Month 2	Otu0033	0.0015	9.00E-06	0.00049	0.005025	7.80E-05	0.001398	0.00999	0.01543909	1083	Corynebacterium(100)	Extremity
Month 2	Otu0023	0.015368	0.002454	0.008036	0.001575	2.20E-05	0.000744	0.017982	0.02658209	1958	Corynebacterium(100)	Face
Month 2	Otu0019	0.018395	0.000617	0.00403	0.006525	0.000374	0.003058	0.018981	0.02688975	3571	Actinomyces(100)	Face
Month 2	Otu0020	0	0	0	0.00745	0.001592	0.006309	0.01998	0.0271728	2581	Clostridium_sensu_stricto(100)	Extremity
Month 2	Otu0013	0.000816	5.00E-06	0.000365	0.01925	0.009254	0.01521	0.022977	0.03004685	5928	Propionibacterium(100)	Extremity

Month 6	Otu0001	0.007625	8.00E-05	0.00141	0.336825	0.134304	0.057945	0.000999	0.00164835	73260	Staphylococcus(100)	Extremity
Month 6	Otu0002	0.254225	0.023266	0.024118	0.071225	0.006845	0.013082	0.000999	0.00164835	59238	Streptococcus(100)	Face
Month 6	Otu0004	0.09245	0.010219	0.015983	0.007225	0.000166	0.002038	0.000999	0.00164835	16867	Prevotella(97)	Face
Month 6	Otu0006	0.070025	0.002215	0.007441	0.0072	0.000113	0.001683	0.000999	0.00164835	12845	Veillonella(100)	Face
Month 6	Otu0007	0.058475	0.001472	0.006066	0.01065	0.000199	0.00223	0.000999	0.00164835	10903	Gemella(100)	Face
Month 6	Otu0009	0.0489	0.001125	0.005304	0.0108	0.000311	0.002791	0.000999	0.00164835	9899	Rothia(100)	Face
Month 6	Otu0010	0.047225	0.002046	0.007152	0.008575	0.000563	0.003752	0.000999	0.00164835	9638	Prevotella(100)	Face
Month 6	Otu0011	0.042525	0.001943	0.00697	0.01175	0.000403	0.003175	0.000999	0.00164835	9591	Actinomyces(100)	Face
Month 6	Otu0013	0.000775	5.00E-06	0.000369	0.0422	0.012416	0.017618	0.000999	0.00164835	7696	Paracoccus(90)	Extremity
Month 6	Otu0015	0.038325	0.001231	0.005546	0.003475	1.70E-05	0.000656	0.000999	0.00164835	6530	Porphyromonas(99)	Face
Month 6	Otu0019	0.02775	0.004834	0.010993	0.001675	5.60E-05	0.001183	0.000999	0.00164835	4811	Simonsiella(100)	Face
Month 6	Otu0020	0.00185	1.00E-05	0.000508	0.02005	0.002534	0.007959	0.000999	0.00164835	4354	Chryseobacterium(100)	Extremity
Month 6	Otu0022	0.024525	0.002298	0.007579	0.00145	1.70E-05	0.000656	0.000999	0.00164835	3309	Soonwooa(100)	Face
Month 6	Otu0023	0.01385	0.000922	0.004802	0.00125	1.40E-05	0.00059	0.000999	0.00164835	2661	Corynebacterium(100)	Face
Month 6	Otu0024	0.00015	0	6.70E-05	0.011875	0.000854	0.00462	0.000999	0.00164835	2608	Bacteroides(100)	Extremity
Month 6	Otu0025	0.000925	2.00E-06	0.000194	0.011475	0.000514	0.003585	0.000999	0.00164835	2301	Kocuria(67)	Extremity
Month 6	Otu0026	0.010325	7.10E-05	0.001328	0.00155	5.00E-06	0.000358	0.000999	0.00164835	2064	Streptococcus(100)	Face
Month 6	Otu0028	4.00E-04	1.00E-06	0.000118	0.009225	0.000599	0.003868	0.000999	0.00164835	1939	Rhizobium(66)	Extremity
Month 6	Otu0030	0.008025	0.000114	0.001692	0.000975	3.00E-06	0.000254	0.000999	0.00164835	1670	Prevotella(100)	Face
Month 6	Otu0032	2.50E-05	0	2.50E-05	0.00565	0.000189	0.002172	0.000999	0.00164835	1215	Bacteroides(100)	Extremity
Month 6	Otu0014	0.000475	1.00E-06	0.000164	0.02845	0.013563	0.018414	0.001998	0.002997	6760	Anaerococcus(100)	Extremity
Month 6	Otu0027	0.00025	1.00E-06	0.000155	0.011075	0.00142	0.005958	0.001998	0.002997	2021	Blautia(100)	Extremity
Month 6	Otu0034	0.00035	1.00E-06	0.000154	0.005825	0.000367	0.003029	0.002997	0.00430004	1213	Brevundimonas(55)	Extremity
Month 6	Otu0003	0.081225	0.002872	0.008474	0.0362	0.008324	0.014426	0.006993	0.00923076	20922	Veillonella(100)	Face
Month 6	Otu0029	0.000125	0	7.30E-05	0.00895	0.001267	0.005628	0.006993	0.00923076	1726	Exiguobacterium(100)	Extremity
Month 6	Otu0018	8.00E-04	3.00E-06	0.000282	0.02695	0.012451	0.017643	0.007992	0.009768	5116	Xanthomonas(96)	Extremity
Month 6	Otu0031	0.000475	2.00E-06	0.000209	0.00605	0.000474	0.003443	0.007992	0.009768	1437	Corynebacterium(100)	Extremity
Month 6	Otu0008	1.00E-04	0	6.00E-05	0.03165	0.020618	0.022704	0.031968	0.03767657	10102	Corynebacterium(100)	Extremity

Table E3: Shannon comparisons based on timepoint, gender, birth method
Highlighted values: p-values <0.05*

Shannon - time

Comparison	p-value
Af-Day 2::Af-Month 2	0.78412628
Af-Month 2::Af-Month 6	0.08969498
Af-Day 2::Af-Month 6	0.03276825
Pf-Day 2::Pf-Month 2	0.00048256
Pf-Month 2::Pf-Month 6	0.3682766
Pf-Day 2::Pf-Month 6	0.00365448
Ch-Day 2::Ch-Month 2	0.10838318
Ch-Month 2::Ch-Month 6	0.02081299
Ch-Day 2::Ch-Month 6	0.00016785
Nt-Day 2::Nt-Month 2	0.13272667
Nt-Month 2::Nt-Month 6	0.00485992
Nt-Day 2::Nt-Month 6	8.20E-05

Shannon - gender

Comparison	p-value
Af Day 2 female::Af Day 2 male	0.71030007
Pf Day 2 female::Pf Day 2 male	0.60267921
Ch Day 2 female::Ch Day 2 male	0.20136937
Nt Day 2 female::Nt Day 2 male	0.20136937
Af Month 2 female::Af Month 2 male	0.88198381
Pf Month 2 female::Pf Month 2 male	0.41189569
Ch Month 2 female::Ch Month 2 male	0.23698524
Nt Month 2 female::Nt Month 2 male	0.06744463
Af Month 6 female::Af Month 6 male	1
Pf Month 6 female::Pf Month 6 male	0.04645154
Ch Month 6 female::Ch Month 6 male	0.3702191
Nt Month 6 female::Nt Month 6 male	0.3702191

Shannon - patient vs. control

Comparison	p-value
Af Day 2 Patient::Af Day 2 Control	0.43587218
Pf Day 2 Patient::Pf Day 2 Control	0.43587218
Ch Day 2 Patient::Ch Day 2 Control	0.52884886
Nt Day 2 Patient::Nt Day 2 Control	0.43587218
Af Month 2 Patient::Af Month 2 Control	0.91179718
Pf Month 2 Patient::Pf Month 2 Control	0.97051246
Ch Month 2 Patient::Ch Month 2 Control	0.43628137
Nt Month 2 Patient::Nt Month 2 Control	0.19031588
Af Month 6 Patient::Af Month 6 Control	0.14314014
Pf Month 6 Patient::Pf Month 6 Control	0.48125095
Ch Month 6 Patient::Ch Month 6 Control	0.63052891
Nt Month 6 Patient::Nt Month 6 Control	0.57874169

Shannon - affected vs. unaffected

Comparison		p-value
Af Day 2		0.47894737
Pf Day 2		0.30526316
Ch Day 2		0.25789474
Nt Day 2		0.30526316
Af Month 2		0.11754386
Pf Month 2		0.76491228
Ch Month 2		0.42647059
Nt Month 2		0.92105263
Af Month 6		0.25789474
Pf Month 6	Month 2	0.41578947
Ch Month 6	Affected::Month	0.84210526
Nt Month 6	2 Unaffected	0.25789474
Af Day 2		0.2749226
Pf Day 2		0.53555212
Ch Day 2		0.75730134
Nt Day 2		0.4377967
Af Month 2		1
Pf Month 2		0.08111455
Ch Month 2		0.12594268
Nt Month 2		0.13480392
Af Month 6		0.31137771
Pf Month 6	Month 6	0.87729618
Ch Month 6	Affected::Month	0.81679567
Nt Month 6	6 Unaffected	0.4377967
Af Day 2		0.24850361
Pf Day 2		0.38472652
Ch Day 2		0.4371517
Nt Day 2		0.61671827
Af Month 2		0.49411765
Pf Month 2		0.55356037
Ch Month 2		0.65441176
Nt Month 2		0.81981424
Af Month 6		0.49411765
Pf Month 6	Month 12	0.81981424
Ch Month 6	Affected::Month	0.89164087
Nt Month 6	12 Unaffected	0.49411765
Af Day 2		0.00980392
Pf Day 2		0.92413632
Ch Day 2		0.24603175
Nt Day 2		0.50280112
Af Month 2		0.92413632
Pf Month 2		0.56629318
Ch Month 2		0.82692308
Nt Month 2		0.92413632
Af Month 6		0.09453782
Pf Month 6	Month 24	0.92413632
Ch Month 6	Affected::Month	0.33590103
Nt Month 6	24 Unaffected	1

Shannon - site

Comparison	p-value
Day 2-Af::Day 2-Pf	0.2942524
Day 2-Af::Day 2-Ch	0.24548721
Day 2-Af::Day 2-Nt	0.23051262
Day 2-Pf::Day 2-Nt	0.02957535
Day 2-Pf::Day 2-Ch	0.01531219
Day 2-Ch::Day 2-Nt	0.72850609
Month 2-Af::Month 2-Pf	0.01207924
Month 2-Af::Month 2-Ch	0.96611786
Month 2-Af::Month 2-Nt	0.67422295
Month 2-Pf::Month 2-Nt	8.20E-05
Month 2-Pf::Month 2-Ch	0.00025177
Month 2-Ch::Month 2-Nt	0.7337265
Month 6-Af::Month 6-Pf	0.00039482
Month 6-Af::Month 6-Ch	0.2942524
Month 6-Af::Month 6-Nt	0.3488102
Month 6-Pf::Month 6-Nt	2.67E-05
Month 6-Pf::Month 6-Ch	3.81E-06
Month 6-Ch::Month 6-Nt	0.47490501

***no p-value correction

Shannon - birth method

Comparison	p-value
Af Day 2 Vaginal::Af Day 2 C-section	0.96346749
Pf Day 2 Vaginal::Pf Day 2 C-section	0.01568627
Ch Day 2 Vaginal::Ch Day 2 C-section	0.14819401
Nt Day 2 Vaginal::Nt Day 2 C-section	0.61671827
Af Month 2 Vaginal::Af Month 2 C-section	0.81981424
Pf Month 2 Vaginal::Pf Month 2 C-section	0.38472652
Ch Month 2 Vaginal::Ch Month 2 C-section	0.19215686
Nt Month 2 Vaginal::Nt Month 2 C-section	0.81981424
Af Month 6 Vaginal::Af Month 6 C-section	0.81981424
Pf Month 6 Vaginal::Pf Month 6 C-section	0.03880289
Ch Month 6 Vaginal::Ch Month 6 C-section	0.24850361
Nt Month 6 Vaginal::Nt Month 6 C-section	0.81981424

Table E4: AMOVA comparisons for skin sites and timepoint

			AMOVA p-values (theta)					
			Month 2	Month 6	Month 12	Month 24		
			Aff::Month 2	Aff::Month	Aff::Month 12	Aff::Month 24		
		Patient::Control	Unaff	6 Unaff	Unaff	Unaff	Male::Female	Vaginal::C-section
	Day 2	0.93	0.313	0.471	0.575	0.979	0.458	0.002*
	Month 2	0.41	0.102	0.974	0.002*	0.418	0.379	0.909
Af	Month 6	0.172	0.039*	0.204	0.281	0.05	0.968	0.168
	Day 2	0.997	0.647	0.251	0.969	0.868	0.252	0.094
	Month 2	0.671	0.424	0.266	0.466	0.093	0.726	0.358
Pf	Month 6	0.361	0.526	0.441	0.377	0.8	0.044*	0.159
	Day 2	0.711	0.811	0.301	0.888	0.432	0.687	0.36
	Month 2	0.544	0.99	0.554	0.533	0.797	0.127	0.283
Nt	Month 6	0.581	0.553	0.648	0.556	0.291	0.511	0.905
	Day 2	0.671	0.369	0.219	0.759	0.263	0.871	0.299
	Month 2	0.689	0.914	0.517	0.602	0.266	0.284	0.78
Ch	Month 6	0.88	0.285	0.834	0.446	0.086	0.548	0.311

***no p-value correction

Fig E1
Rarefaction curves for sampling at each site to a cutoff of 1000 sequences; OTUs calculated at a cutoff of 97% nucleotide similarity. Each point represents mean ± SEM for all subjects at the site and sampling time indicated.

Fig E2
Relative abundance of major taxa; each bar represents a subject sampled at a single site and time point.

Fig E3
All samples month two and month six clustered by principal coordinates analysis based on theta similarity coefficients. At month two, Af and Pf had similar centroids (AMOVA p-value=0.18), as did Ch and Nt (p=0.276), and each still clustered distinctly from the other site pair ($p < 0.006$). At month six, Af and Pf had distinct centroids (AMOVA p=<0.006), but Ch and Nt clustered together (p=1); and the two site pairs clustered distinctly ($p < 0.006$).
Post-hoc p-values adjusted with Bonferonni correction (n=6)

Fig E4
Mean of relative abundance of major taxa; each bar represents the mean ± SEM of all subjects at a single site and time point.

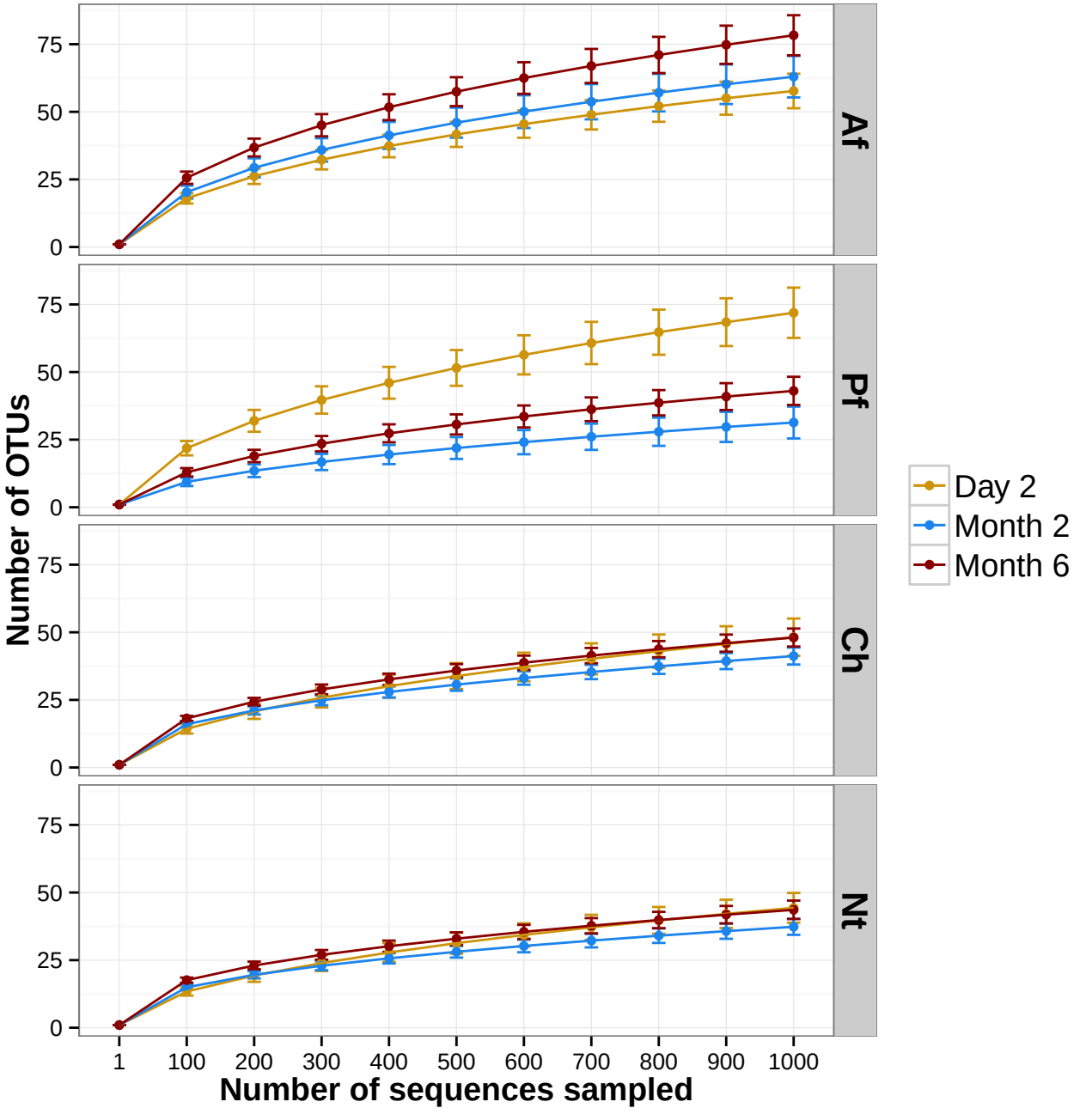
Fig E5
(a) Popliteal fossa samples clustered by principal coordinates analysis based on theta similarity coefficients. Using AMOVA, samples clustered significantly between day two and month six ($p = 0.003$), between day two and month six ($p = 0.042$), but not between month two and month 6 (p=1).
(b) Nasal tip samples clustered by principal coordinates analysis based on theta similarity coefficients. Using AMOVA, samples clustered distinctly between day two and month two (p=<0.003), between day two and month six(p=<0.003), and between month two and month six (p=0.06).

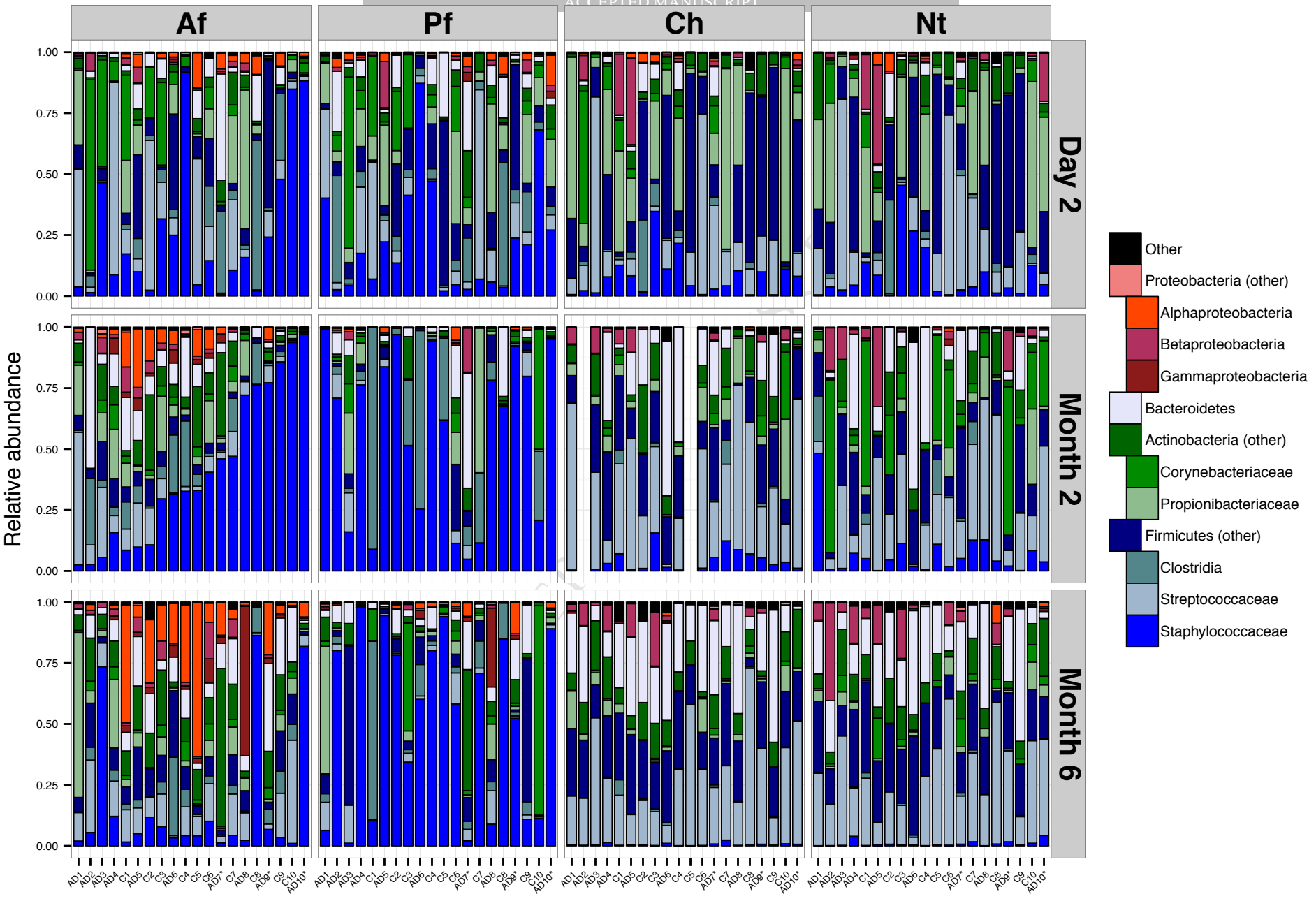
Post-hoc p-values adjusted with Bonferonni correction (n=3)

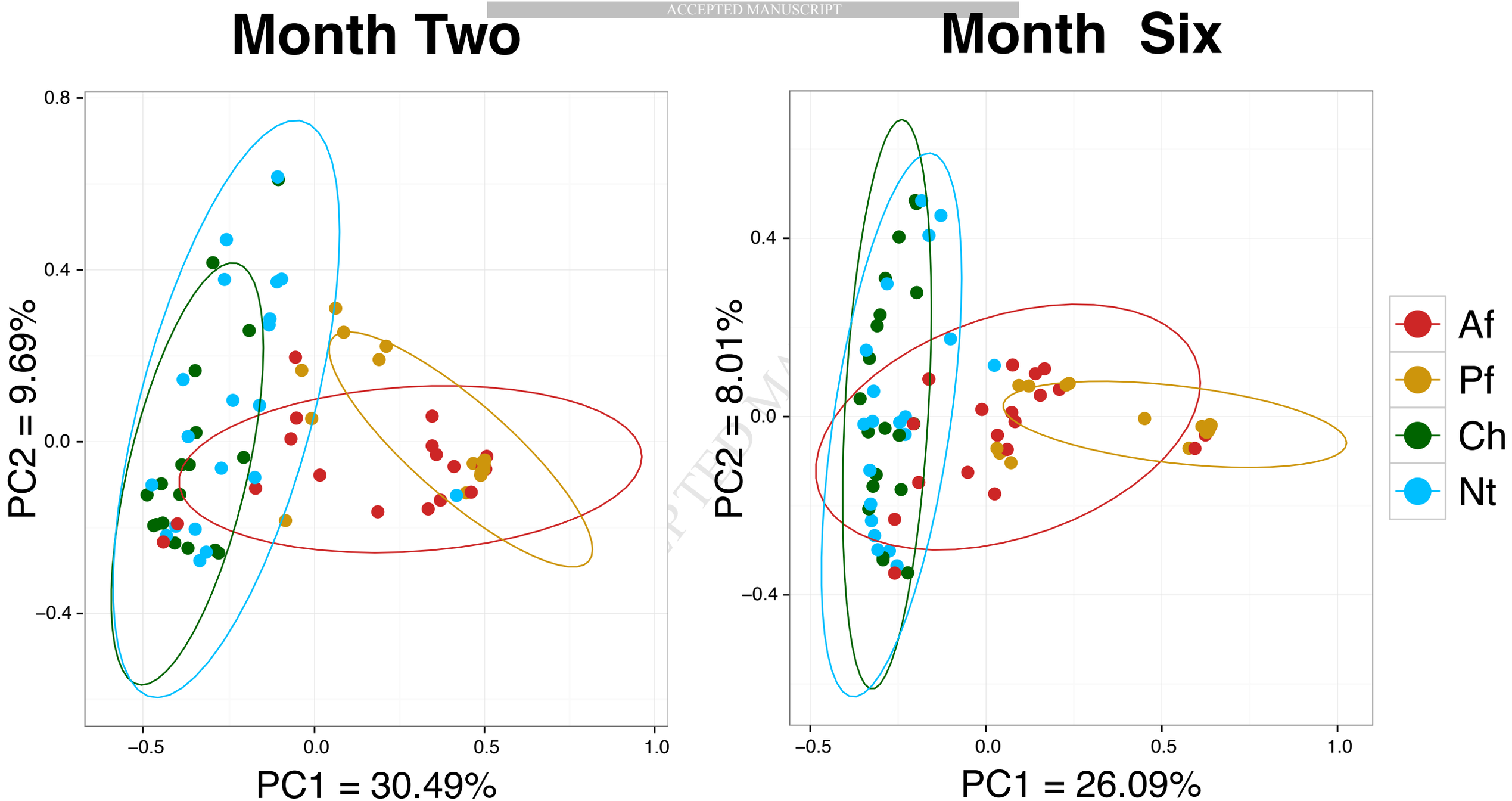
Fig E6
Relative abundance of Staphylococcal species; each bar represents a subject sampled at a single site and time point.

Fig E7
Mean of relative abundance of Staphylococcal species; each bar represents the mean ± SEM of all subjects at a single site and time point.

Fig E8
(a) Day 2 antecubital fossa samples clustered by principal coordinates analysis based on theta similarity coefficients. Samples clustered by birth method (AMOVA p-value = 0.005)
(b) Day 2 cheek samples by principal coordinates analysis of theta similarity coefficient. Samples did not cluster by birth method (AMOVA p-value = 0.337).
(c) Shannon diversity was generally similar between the birth methods, with only the popliteal fossa significantly different at day two (Wilcox rank-sum test p-value = 0.016)


Af
Pf
Ch
Nt
Number of OTUs
0
25
50
75
Number of sequences sampled
1
100
200
300
400
500
600
700
800
900
1000
Day 2
Month 2
Month 6





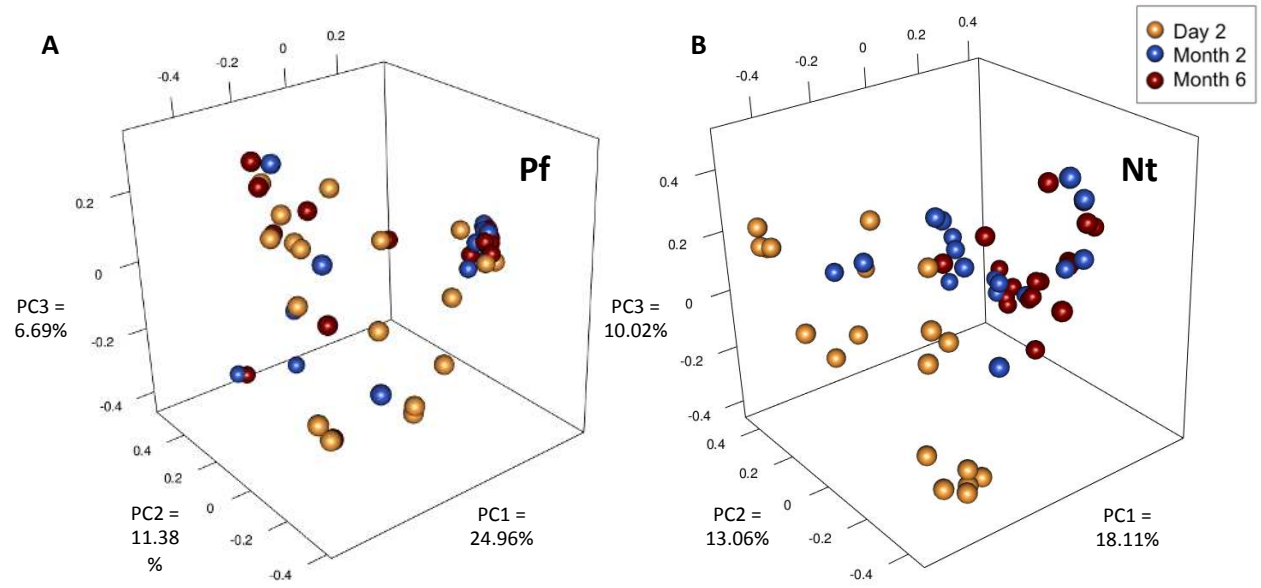
Mean proportion

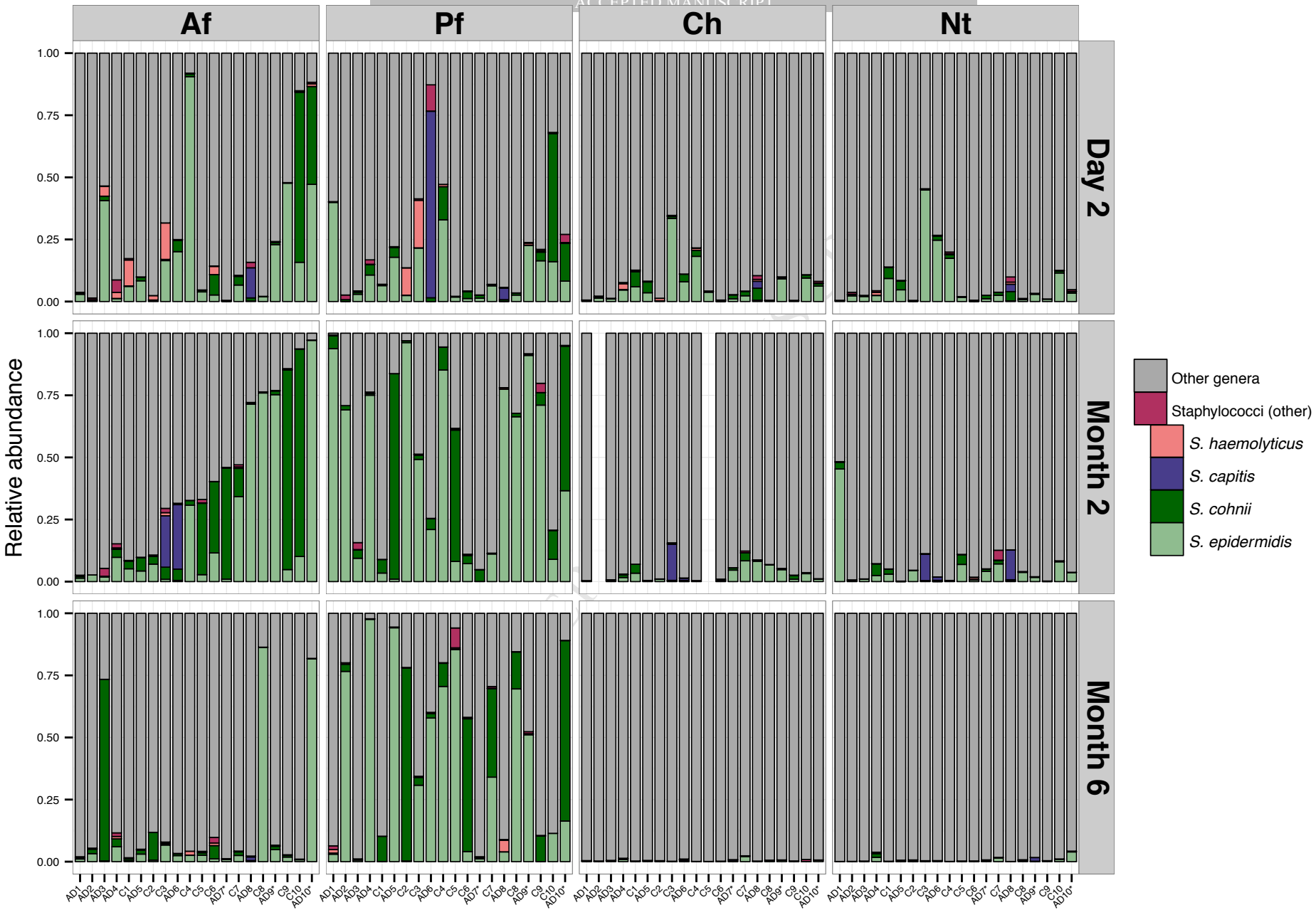
0.0
0.2
0.4
0.6

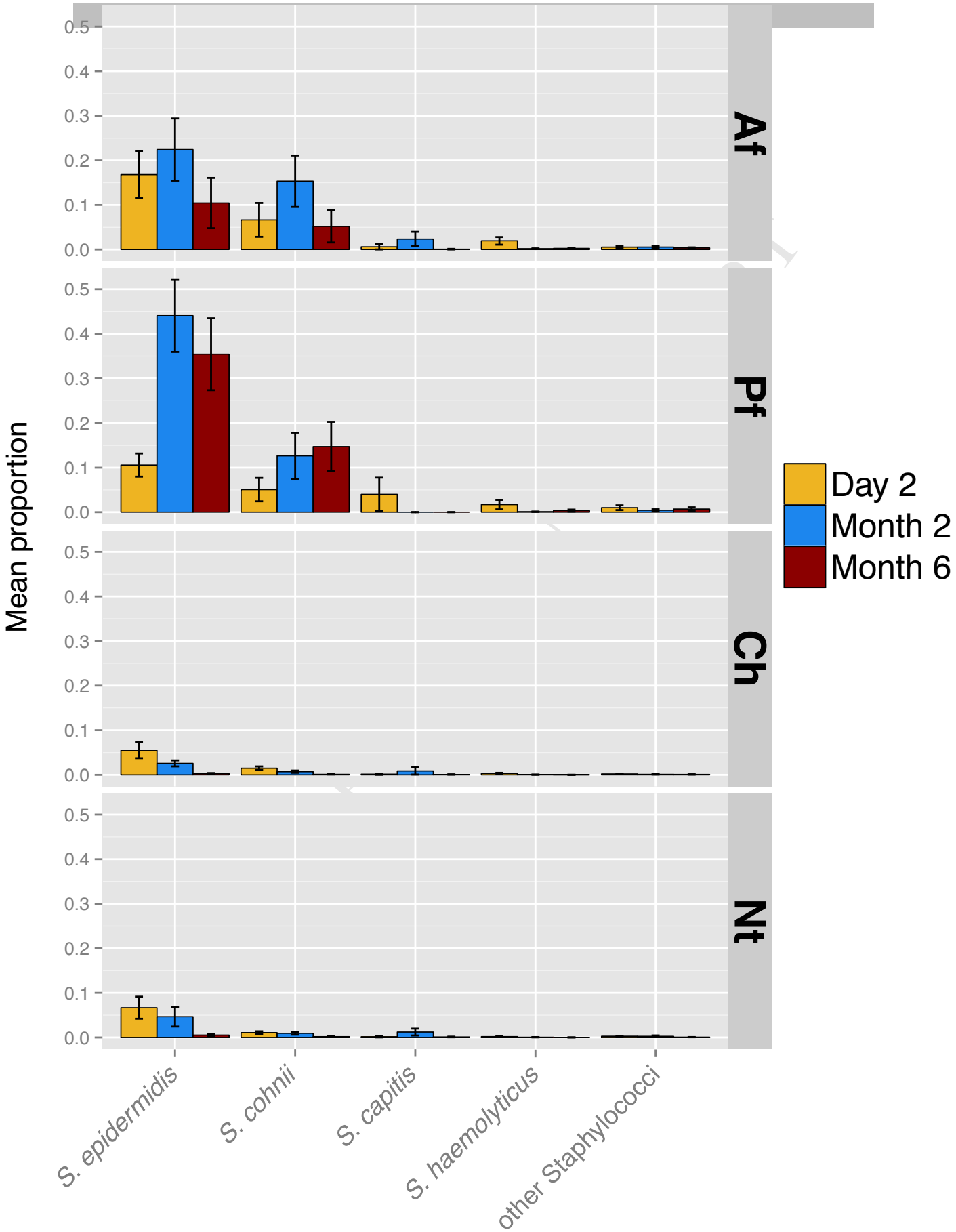
Af
Pf
Ch
Nt

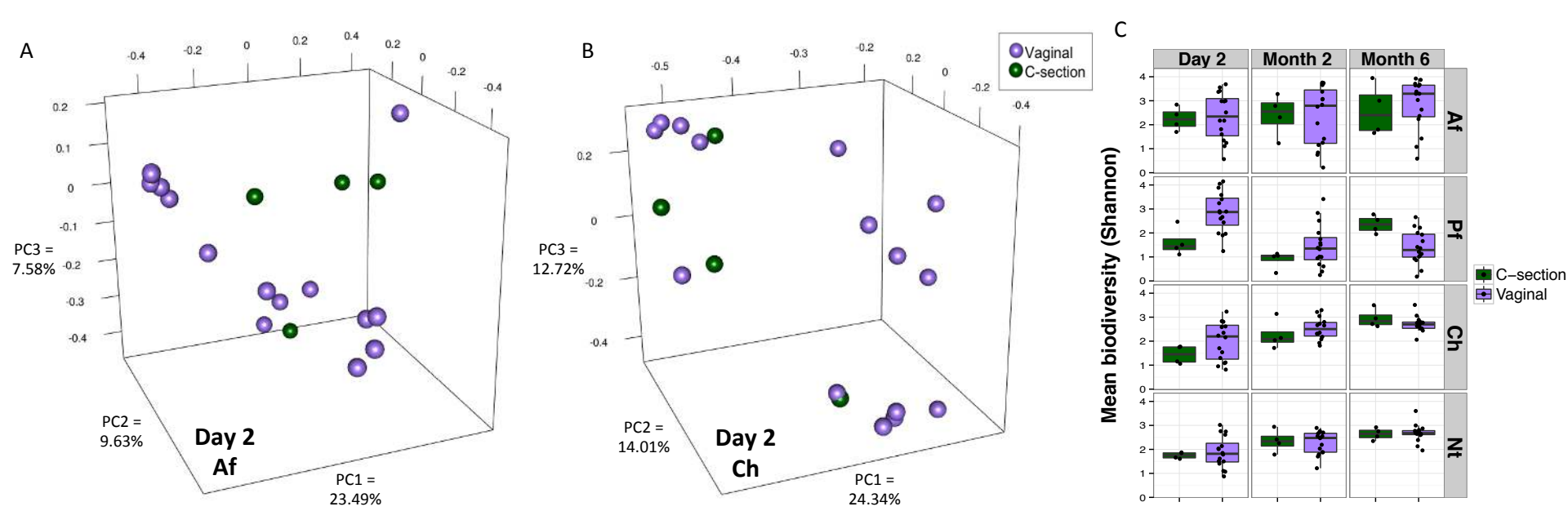
Day 2
Month 2
Month 6

Staphylococcaceae
Streptococcaceae
Clostridia
Firmicutes (other)
Propionibacteriaceae
Corynebacteriaceae
Actinobacteria (other)
Bacteroidetes
Gammaproteobacteria
Betaproteobacteria
Alphaproteobacteria
Proteobacteria (other)
Other


A
Pf
PC3 = 6.69%
PC2 = 11.38 %
PC1 = 24.96%
B
Nt
PC3 = 10.02%
PC2 = 13.06%
PC1 = 18.11%
Day 2
Month 2
Month 6




Mean proportion
0.5
0.4
0.3
0.2
0.1
0.0
Af
Pf
Ch
Nt
Day 2
Month 2
Month 6
S. epidermidis
S. cohnii
S. capitis
S. haemolyticus
other Staphylococci


A
PC3 =
7.58%
PC2 =
9.63%
Day 2
Af
PC1 =
23.49%
B
Vaginal
C-section
PC3 =
12.72%
PC2 =
14.01%
Day 2
Ch
PC1 =
24.34%
C
Day 2
Month 2
Month 6
Af
Pf
Ch
Nt
Mean biodiversity (Shannon)
C–section
Vaginal